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BIOCOMPATIBILITY OF ACELLULAR DERMAL MATRIX GRAFT EVALUATED IN CULTURE OF MURINE MACROPHAGES

AVALIAÇÃO DA BIOCOMPATIBILIDADE DA MATRIZ DÉRMICA ACELULAR EM CULTURA DE CÉLULAS

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ABSTRACT

The acellular dermal matrix allograft has been used as an alternative to autogenous palatal mucosal graft. The aim of this study was the evaluation of the biocompatibility of an acellular dermal matrix (AlloDerm®) in culture of macrophages. For hydrogen peroxidase determination we used the method of Pick & Kesari, and the Griess method for nitric oxide determination,. Statistical analysis showed no significant difference ($p \leq 0,05$) in the release of nitric oxide and hydrogen peroxide by the macrophages exposed to acellular dermal matrix and the negative control. The results suggest that acellular dermal matrix did not activate the cell inflammatory response.
Uniterms: Dermal allograft; Macrophages; Nitric oxide; Hydrogen peroxide.

RESUMO

A matrix dérmica acelular tem sido utilizada como alternativa para a substituição de enxerto gengival autógeno. O objetivo deste estudo foi avaliar a biocompatibilidade em cultura de células de macrófagos da matriz dérmica acelular (AlloDerm®). Foram utilizados os métodos de Pick & Kesari, para a determinação da presença de peróxido de hidrogênio (H_2O_2) e de Griess para a determinação de ácido nitroso (NO). Não houve diferença estatisticamente significante ($p \leq 0,05$) no aumento da presença de NO e H_2O_2 quando macrófagos foram expostos na presença da matrix dérmica acelular quando comparado com o controle negativo. Pode-se concluir que a matrix dérmica acelular é biocompatível aos tecidos humanos.
Unitermos: Matriz dérmica; Macrófagos; Ácido nítrico; Peróxido de hidrogênio.

INTRODUCTION

Several techniques have been developed to solve gingival aesthetic problems. The techniques can be grouped as pediculated grafts and autogenous grafts (epithelial free grafts and connective tissue graft)[16,22]. Aiming at reducing the problems with these treatments, new techniques and materials have been developed[6,9,18-22,24,26,31].

A recent material developed for soft tissue grafting was the acellular dermal matrix (AlloDerm®, Lifecore Biomedical, Oral Restorative Division, Chaska,MN)[1,6,25,30,32]. This allograft material has advantages to reduce surgical complications, decrease donor site discomfort and improve esthetic tissue. This graft is obtained from an allograft donor skin and produced by a carefully controlled process that removes the epidermis and dermis cells without altering the extracellular matrix structure, reducing the possible immune response and the transmission of diseases.

The need for biocompatible materials implies the necessity of in vitro toxicity tests, animal experimentation usage tests and clinical studies in humans[23].The biological system used in in vitro cytotoxicity testing of some materials

are cells in culture. Thus, culture of macrophage cells can be used to evaluate the biocompatibility of materials. These cells participate in many aspects of host defense, inflammation and immunity, partly through their ability to undergo adaptive responses to the conditions or stimuli that prevail at sites to which they have been attracted[2].

Among the numerous secretory products of macrophages there are two groups of inorganic compounds with a high degree of chemical reactivity: the ROI (including super oxide, hydrogen peroxide, and in some populations of mononuclear phagocytes, the products of myeloperoxidase), and the RNI including nitrite (NO_2^-) and highly related reactive oxides such as nitric oxide and nitrogen dioxide[3].Macrophage-derived RNI are of interest for at least three reasons: their production is under strict immunological control[27,28], they are synthesized by enzymes novel to mammalian biochemistry, which have not been yet well characterized[13], and they appear to play an important role in some of the carcinogenic[13,15], antitumor[12,29] and antimicrobial[7] actions of the activated macrophage.

Although Alloderm® is tested before being released for sale; we assume that it is important to evaluate the biocompatibility in macrophages culture.

MATERIAL AND METHODS

This study was approved by the Institutional Committee on Animal Research, School of Pharmaceutical Science.

Animals

The Animals Laboratory of the School of Pharmaceutical Science, UNESP (State University of São Paulo) Araraquara, SP, BRAZIL supplied six-week old male Swiss mice weighing 18 to 25 g.

Acellular dermal matrix samples

Samples of acellular dermal matrix (ADM), commercially known as AlloDerm®, measuring 4x4 mm, from different batches were obtained at the Department of Periodontology of Araraquara Dental School UNESP-Araraquara, São Paulo, Brazil.

Cell macrophage

Mice were injected i.p. 3 to 4 days before harvesting with 3 ml of thioglycollate broth. Macrophages were obtained after killing the mice with chloroform, and the peritoneum was exposed using sterile scissors. Saline solution (0.85% NaCl) was introduced into the peritoneum and after digital massage, the suspension was removed by aspiration. This suspension of peritoneal cells and saline was placed in a Neubauer chamber and counted in order to obtain the ideal concentration for each test. The adherent cells of PEC were re-suspended in RPMI-1640 medium at concentration of 5_10^6 cells/ml, and 100ll of this suspension was added to each well tissue dish along with 100 ll of a 10.0lg/ml (1.0lg) E. coli O111B lipopolysaccharide (LPS) solution was used as positive control, or RPMI-1640 medium as negative control. The cells were incubated for 24 h before stimulation, and 50 ll aliquots of culture supernatant were mixed with 50ll of Griess reagent (1% w/v sulphanylamide, 0.1% w/v naphthylethylenediamine and 3% H_3PO_4), incubated at room temperature for 10 min, and the color reaction was determined at 450 nm with a Multiskan Ascent ELISA reader (Labsystems, Helsinki, Finland) Supernatants from quadruplicate cultures were assayed in four experiments and reported as the mean NO_2 concentration ±SD.

NO_2 release

Nitrite concentration in the medium was measured by a micro plate assay method[5]. The Alloderm® was incubated with cell suspension in a concentration of $5x10^6$ cells / ml, at 37° C and atmosphere with CO_2 5%. After 24 hs, 50mL aliquots were removed from conditioned medium and incubated with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihidrochloride, 0.25% H_3PO_4) at room temperature for 10 minutes. The absorbance at 550 nm was determined in a micro plate reader. The tests were made in quadruplicate and the results were expressed in micromoles of NO / 5 x 10^5 peritoneal cells, from a standard curve established in each test, constituted of known molar concentrations of NO in RPMI – 1640 medium.

H_2O_2 release

The method depends on the determination of hydrogen peroxide (H_2O_2) release in the culture of peritoneal macrophages from Swiss mice. Suspensions of peritoneal cells were performed using a concentration of 2 x 10^6 cells/ml in a solution of phenol red, containing 140 mM NaCl, 10 mM potassium phosphate, pH 7.0; 5.5 mM dextrose; 0.56 mM phenol red and type II horseradish peroxidase 0.01 mg/mL (Sigma). Aliquots of 0.1 m were transferred to culture plates, flat bottomed containing 96 wells (Corning). The acellular dermal matrix or 50 mL of Zymosan solution (5 mg/ml, Sigma) were added to each well. The samples were incubated for one hour at 37°C in a 5% CO_2 atmosphere. After the period of incubation, the reaction was interrupted by addition of 10 mL of NaOH 4N. Experiments were done in quadruplicate and the absorbance was determined in an ELISA automatic photometer, with a 620 nm filter. The results were expressed in nanomols of H_2O_2/2 x 10^5 peritoneal cells, from a standard curve established in each test, constituted of known molar concentrations of H_2O_2 in buffered phenol red.

Statistical Analysis

Comparisons between groups were performed using Student's T test. P values <0.05 were considered statistically significant.

RESULTS

Figures 1 and 2 illustrate the release of nitric oxide and hydrogen peroxide when macrophages were exposed to

acellular dermal matrix, respectively. Statistical analysis showed no significant difference ($p \leq 0.05$) when compared with negative control. However, the level of release of these mediators in positive control was statistically different ($p \geq 0.05$).

DISCUSSION

This study evaluated the cell inflammatory response of in vitro macrophages in contact with the acellular dermal matrix measuring the release of nitric oxide and hydrogen peroxide. The positive control using zymosan-SIGMA to H_2O_2 release and LPS-SIGMA to NO release showed a high potential of macrophages activation and liberation of H_2O_2 and NO, respectively. The acellular dermal matrix was not able to stimulate macrophage liberation of hydrogen peroxide (H_2O_2) and nitric oxide (NO).

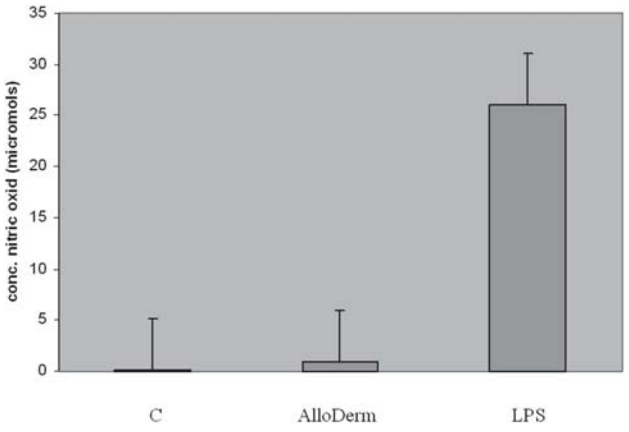

FIGURE 1- Production of nitric oxide by peritoneal macrophages cultured with medium (C), acellular dermal matrix and lypopolysaccharide (1x10^3 mg/well), by 24 hours of incubation at 37 degree. The results were expressed as the mean +/- SD of quadruplicate from five determinations

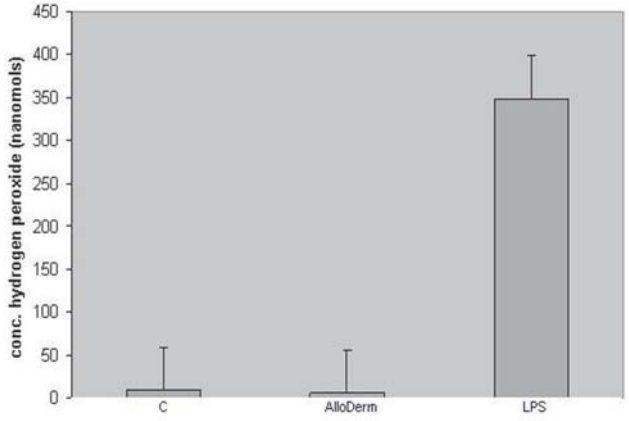

FIGURE 2- Production of hydrogen peroxide by peritoneal macrophages cultured with medium, acellular dermal matrix and Zymosan (250µg/well). In the test phenol red was used as indicator of presence of hydrogen peroxide. The results were expressed as the mean +/- SD of quadruplicate from five determinations

In respect to these results we pointed out that Liversey, et al.[14] (1994) evaluating immunohistochemically the material AlloDerm® did not find any antigen that could develop an immune reaction.

Cellular events were evaluated in some human studies with ADM grafts for root coverage[4,10,11,33]. In 1998, Harris[10], using acellular dermal matrix graft in treatment of gingival recessions in patients, obtained complete root coverage on two of three defects. In histological analysis, the AlloDerm® had incorporated and became part of the gingival tissue in the area. The same author[11] in 2001 reported a comparative clinical study of root coverage obtained with ADM versus connective tissue. Biopsy of the grafted area revealed elastin fibers. The author stated that the presence of these fibers implied that the ADM was being incorporated into the host tissue.

In a recent study Cummings, et al.[4] (2005) histologically evaluated the acellular dermal matrix graft. The findings of the study showed new fibroblast, vascular elements, and collagen were present throughout the ADM, with retention of the transplanted elastin fibers.

All of these results can be directly related with the laboratory processing of human skin obtained in tissue banks with the removal of all cells without altering the connective tissue structure, composed of type I collagen fibers[14]. In this respect, the AlloDerm® matrix is rigorously controlled by the FDA according to the guidelines of the American Association of Tissue Banks. Apart from the selection of possible donors, serologic and microbiologic exams are also performed to screen for diseases such as AIDS, syphilis, hepatitis, etc. The patented process of preparation and lyophilization removes all cells, preserving the collagen structure of the connective tissue. In the manufactured controls, histological and immunohistochemistry tests are carried out to check the complete removal of all cellular components.

Within the limits of this study the present results show that there was no release of hydrogen peroxide (H_2O_2) and nitric oxide (NO), suggesting that the acellular dermal matrix did not activate the cell inflammatory response, although new studies should be accomplished with Alloderm® use.

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